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(54) Title: ANTI-ATHEROSCLEROTIC AND ANTI-THROMBOTIC AGENT AND THE USE THEREOF

(57) Abstract

A pharmaceutical agent for the prevention or treatment of any of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity, is provided. The agent comprises a short chain fatty acid, or a pharmaceutically acceptable salt, derivative or precursor thereof, in a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but digestible or soluble in the colon of a mammal. Preferably the agent comprises calcium acetate in a shellac coating.

Anti-Atherosclerotic and Anti-Thrombotic Agent and the Use Thereof

INTRODUCTION AND BACKGROUND TO THE INVENTION

This invention relates to a pharmaceutical agent for the prevention or treatment of any of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot

5   lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity and the use thereof.

It is generally known that atherosclerosis is primarily caused by increased levels of cholesterol in human beings and that thrombosis is caused by the

10   polymerisation of fibrin to form fibrin clots.

Low density lipoprotein cholesterol (LDL-C), occurring in relatively high concentrations, is particularly responsible for an increase in cardiovascular disease, especially when the LDL-C is oxidised by free radicals such as lipid

15   peroxides. Although it is has been reported that dietary fibre can modify lipid metabolism in man, no effects of fibre, fibre components or metabolites thereof on lipid peroxidation have been reported.

It is further known that fermentable non-starch polysaccharides such as

20   pectin, are fermented in the colon of a mammal to short chain fatty acids or derivatives thereof, such as acetate, propionate and butyrate. The butyrate is absorbed by the colon cells while the propionate and acetate

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move to the liver. The propionate is retained in the liver while the acetate

is distributed throughout the cells and plasma of the mammal.

OBJECT OF THE INVENTION

5   It is an object of the present invention to provide a novel pharmaceutical

agent for the prevention or treatment of any of the following conditions in

mammals: atherosclerosis, thrombosis, unwanted high levels of free

radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot

characteristics, unwanted high levels of free fatty acids and obesity and the

10   use thereof.

SUMMARY OF THE INVENTION

According to the invention a pharmaceutical agent for the prevention or

treatment of any of the following conditions in mammals: atherosclerosis,

15   thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot

lysis times, unwanted fibrin clot characteristics, unwanted high levels of

free fatty acids and obesity, is provided which comprises a short chain fatty

acid, or a pharmaceutically acceptable salt, derivative or precursor thereof,

in a pharmaceutically acceptable protective coating which is resistant to

20   digestion and solution in the stomach and small intestine of a mammal, but

digestible or soluble in the colon of a mammal.

Preferably the pharmaceutically acceptable salt of the short chain fatty acid is the calcium salt thereof.

Preferably the short chain fatty acid comprises acetic acid.

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The protective coating may comprise a natural or synthetic resin such as shellac.

The pharmaceutical agent preferably comprises calcium acetate in the form

10   of a capsule, tablet or pill coated with such a resin.

Preferably the agent comprises between 0,1 grams and 100,0 grams of the acetate.

15   According to another aspect of the invention a method for the treatment or prevention of any one or more of said conditions in a mammal includes the step of administering to the colon of a mammal an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof.

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Preferably the agent is administered to the colon, via the digestive track of the mammal.

According to another aspect of the invention there is provided the use of an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof in a method for the treatment or prevention of any one or more of said conditions in mammals.

According to another aspect of the invention there is provided the use of an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof, in the manufacture of a medicament for use in a method for the treatment or prevention of any one or more of said conditions in mammals.

Further according to the invention, the aforesaid method includes the step of administering the agent orally in the form of a capsule, pill or tablet coated with a protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but soluble or digestible in the colon of said mammal.

Still further according to the invention the pharmaceutically acceptable salt is the calcium salt of the short chain fatty acid.

Still further according to the invention the short chain fatty acid is acetic acid.

Still further according to the Invention the protective coating comprises a natural or synthetic resin such as shellac.

5   Applicant has found that the aforesaid clinical effects can be attained by administering the agent to a human being in an amount of between 0,1 gram and 100,0 gram at least once a day.

SPECIFIC DESCRIPTION OF THE INVENTION

The invention will now be described further by way of the following non-
10   limiting examples.

The codes used in the examples denote the following:

ApoA	--	APO-PROTEIN A
ApoB	--	APO-PROTEIN B
BMI	--	BODY MASS INDEX = WEIGHT/(LENGTH)^2
DBP	--	DIASTOLIC BLOOD PRESSURE
FFA	--	FREE FATTY ACIDS
FFA/ALB	--	FREE FATTY ACID TO ALBUMIN RATIO
HAEMATOCRIT	--	% PACKED CELLS IN BLOOD
HDL-C	--	HIGH DENSITY LIPOPROTEIN CHOLESTEROL
IR	--	INSULIN RESISTANCE
LDL-C	--	LOW DENSITY LIPOPROTEIN CHOLESTEROL

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LP(a)   --   LIPOPROTEIN (a)

MPC   --   MACROMOLUCULAR PROTEIN COMPLEX

SBP   --   SYSTOLIC BLOOD PRESSURE

TBARM   --   THIOBARBITURIC REACTIVE SUBSTANCES OF

5   MALONDEALDEHYDE

TC   --   TOTAL CHOLESTEROL

TG   --   TRIGLYCERIDES

TP   --   TOTAL PROTEIN

μT   --   MASS LENGTH RATIO FROM TURBIDITY

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EXAMPLE 1

The respective effects of pectin and an acetate when administered to the colon of a mammal were determined during a first experiment. The experiment was conducted in the following two phases:

5

```
            Week 0              ┌─────────────────┐
         ┌──────────────────────│ Baseline Phase I │─────────────────────┐
         │                      │  - blood 1 & 2   │                     │
         │                      └─────────────────┘                     │
         │                                                              │
      Pectin          ┌───────────────────────────────┐           Cornflour
      n=10            │ Week 2 : weight measurement   │            n=10
         │            └───────────────────────────────┘              │
         │                   ┌─────────────────────┐                 │
         └───────────────────│ End of supplementation │──────────────┘
            Week 4           │    - blood 3 & 4     │
                             └─────────────────────┘
                                        │
                             ┌─────────────────────┐
            Week 8           │ Baseline Phase II    │
         ┌───────────────────│    - blood 5 & 6     │──────────────────┐
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      Acetate         ┌───────────────────────────────┐            Pectin
      n=10            │ Week 10 : weight measurement  │            n=10
         │            └───────────────────────────────┘              │
         │                   ┌─────────────────────┐                 │
         └───────────────────│ End of supplementation │──────────────┘
            Week 12          │    - blood 7 & 8     │
                             └─────────────────────┘
```
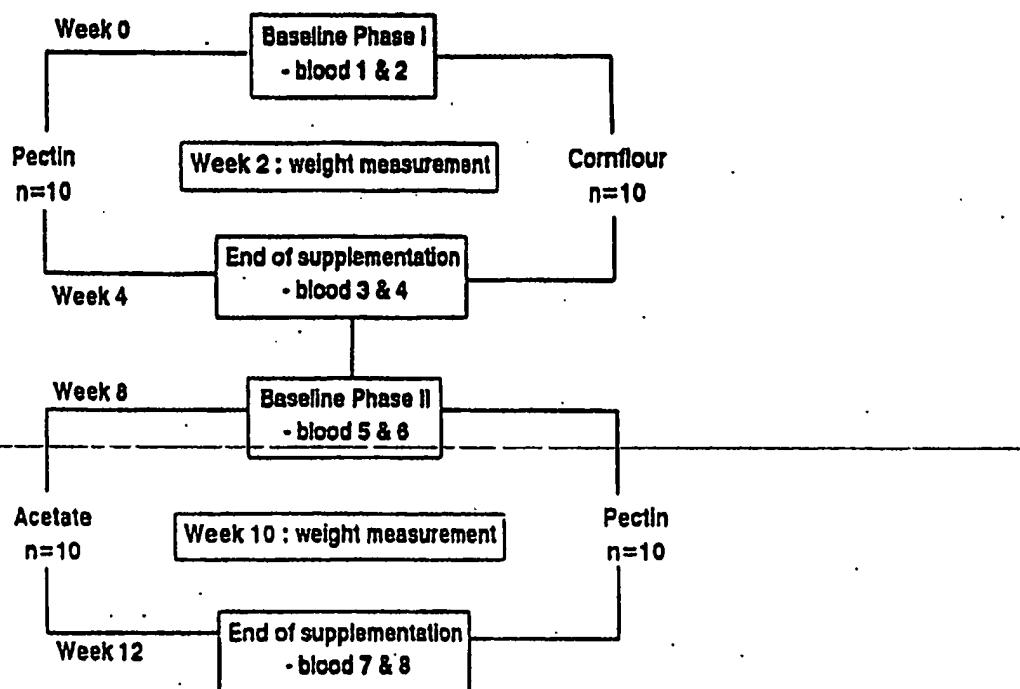
20

Twenty human males participated in the experimentation and these subjects were not on any medication for any chronic diseases at the time, and also had no history of cardiovascular disease. All the subjects were at the time following a relatively high fibre, low fat diet. During the first phase ten

5   subjects consumed a total of 15 grams of pectin per day in four aliquots, while the other ten consumed a total of 15 grams of placebo (starch) per day in four aliquots.

During the second phase, the first group consumed a total of 7,5 grams of

10   calcium acetate per day in four aliquots and the second group consumed a total of 15 grams of pectin per day in four aliquots. The calcium acetate was administered in capsules which were coated with a protective coating comprising a resin known commercially as shellac. This protective coating is resistant to digestion and solution in the stomach and small intestines,

15   but not resistant to the enzymes of the organisms usually found in the colon, so that the calcium acetate was thus released in the colon. Details of the subjects are given in Table 1.

TABLE 1:   PERSONAL DETAILS OF SUBJECTS PARTICIPATING IN THE

EXPERIMENTATION

VARIABLE	PECTIN : PHASE 1 ACETATE : PHASE 2	PLACEBO : PHASE 1 PECTIN : PHASE 2
SEX	Male	Male
AGE (years)	45.27 ± 12.24	42.0 ± 10.22
SBP (mmHg)	125.9 ± 9.7	125.0 ± 14.3
DBP (mmHg)	81.3 ± 9.77	79.5 ± 10.1
Activity level	Medium	Medium
Cardiovascular events	No history	No history
WEIGHT (kg)	89.50 ± 11.81	92.10 ± 15.03
BMI (kg^1m^{-2})	27.50 ± 2.99	29.70 ± 3.09
MEDICATION	None	None

Blood samples where taken from the subjects after each phase and a large

number of variables where tested. The results of these tests are given in.

Tables 2 to 5.

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Table 2   Means and standard deviations of body weight
          and BMI changes

VARIABLE	PHASE 1				PHASE 2			
	PECTIN		PLACEBO		PECTIN		ACETATE	
	BASELINE	END	BASELINE	END	BASELINE	END	BASELINE	END
BODY WEIGHT (kg)	89,50 ± 11,81	89,10 ± 11,92	92,10 ± 15,03	92,10 ± 15,54	92,07 ± 15,54	91,55 ± 14,55	88,16 ± 12,35	83,04 ± 10,80
BMI (kg/m²)	27,50 ± 2,99	27,40 ± 2,98	29,70 ± 3,09	29,50 ± 3,04	29,46 ± 3,03	29,32 ± 2,82	26,90 ± 2,82	25,65 ± 2,62

15

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Table 3 Means and standard deviations of baseline and end of supplementation haemorheological and haemostatic variables

VARIABLE	PHASE 1				PHASE 2			
	PECTIN		PLACEBO		PECTIN		ACETATE	
	BASELINE	END	BASELINE	END	BASELINE	END	BASELINE	END
HAEMATOCRIT (%)	48.70 ±2.45	48.10 ±2.69	48.70 ±2.45	48.10 ±2.64	47.38 ±2.67	45.39* ±2.55	48.55 ±1.88	46.83* ±1.85
HAEMOGLOBIN (mmol/l)	10.30 ±1.09	9.60* ±0.98	10.30 ±0.91	10.60 ±0.98	10.82 ±1.07	9.75* ±0.84	11.12 ±0.34	10.48 ±0.50
VISCOSITY (cP)	1.81 ±0.08	1.60* ±0.19	1.80 ±0.09	1.70* ±0.07	1.75 ±0.10	1.62* ±0.13	1.92 ±0.22	1.61* ±0.22
COMPACTION (%)	21.51 ±3.65	30.16* ±4.41	21.60 ±3.85	24.63 ±3.47	20.67 ±5.86	31.93* ±6.09	22.47 ±2.90	32.21* ±9.15
P (Del/cm x 10^..)	19.94 ±6.27	24.80* ±4.22	19.80 ±5.96	19.10 ±10.49	19.02 ±11.93	32.10* ±7.52	22.93 ±10.41	34.28* ±5.42
PERMEABILITY (x 10^.. cm²)	279.58 ±101.16	336.25* ±119.06	275.5 ±116.4	307.09 ±72.98	131.18 ±99.94	285.36* ±84.50	212.52 ±76.32	306.81* ±80.83
LYSIS TIME (c50%)	205.6 ±16.13	232.9* ±17.9	205.5 ±14.9	221.4 ±10.9	285.6 ±16.13	132.9* ±17.9	251.9 ±10.7	130.3* ±14.8
MPC (g/l)	0.1218 ±0.0394	0.0836* ±0.0395	0.109 ±0.083	0.097 ±0.059	0.1002 ±0.029	0.0807* ±0.0314	0.1146 ±0.0429	0.0852** ±0.0371
CLOT (FIBRIN) (g/l)	2.22 ±0.47	1.90* ±0.37	2.30 ±0.44	2.10 ±0.33	2.55 ±0.70	1.86* ±0.37	2.00 ±0.28	1.62* ±0.16
FIBRINOGEN (g/l)	3.51 ±0.62	3.30 ±0.48	3.60 ±0.62	3.62 ±0.35	4.11 ±0.90	3.72 ±0.62	4.10 ±2.44	3.64 ±0.91

Table 4. Means and standard deviations of baseline and end of supplementation lipid variables

VARIABLE	PHASE 1				PHASE 2			
	PECTIN		PLACEBO		PECTIN		ACETATE	
	BASELINE	END	BASELINE	END	BASELINE	END	BASELINE	END
TC (mmol/1)	6.50 ± 0.27	5.67* ± 0.48	6.60 ± 0.97	6.40 ± 0.79	6.89 ± 0.86	6.07 ± 0.79	6.55 ± 0.63	5.81* ± 0.49
LDL-C (mmol/1)	4.70 ± 0.35	4.10* ± 0.59	4.80 ± 0.98	4.60 ± 0.63	5.17 ± 0.60	4.59 ± 0.69	4.97* ± 0.53	4.20* ± 0.38
HDL-C (mmol/1)	1.20 ± 0.18	1.03* ± 0.14	1.20 ± 0.19	1.10 ± 0.26	0.92 ± 0.01	1.13* ± 0.27	1.11 ± 0.14	1.18 ± 0.11
% HDL-C (%)	18.30 ± 3.07	18.20* ± 2.64	17.70 ± 2.29	17.30 ± 3.40	15.46 ± 0.01	18.79* ± 4.48	17.04 ± 0.69	20.32* ± 2.98
H₂O₂ (µM)	1.70 ± 0.76	0.84* ± 0.38	1.50 ± 0.52	1.45 ± 0.78	1.20 ± 0.33	0.73* ± 0.23	1.27 ± 0.48	0.81* ± 0.22

VARIABLE	PHASE 1				PHASE 2			
	PECTIN		PLACEBO		PECTIN		ACETATE	
	BASELINE	END	BASELINE	END	BASELINE	END	BASELINE	END
ApoA (mmol/1)	1.60 ± 0.14	1.23* ± 0.12	1.50 ± 0.18	1.40* ± 0.22	1.53 ± 0.18	1.39* ± 0.22	1.50 ± 0.16	1.40* ± 0.15
ApoB (mmol/1)	1.70 ± 0.15	1.29* ± 0.12	1.70 ± 0.28	1.50* ± 0.18	1.77 ± 0.65	1.39* ± 0.16	1.47 ± 0.15	1.34* ± 0.14
TG (mmol/1)	2.00 ± 0.84	1.78 ± 0.64	2.10 ± 0.98	2.00 ± 0.64	1.99 ± 0.59	1.78 ± 0.39	0.65 ± 0.59	1.33* ± 0.33
LP(a) (mmol/1)	349.23 ± 317.37	251.93* ± 213.27	281.0 ± 142.08	249.33 ± 129.33				
TBARM (µM)	0.60 ± 0.59	0.30 ± 0.20	0.50 ± 0.25	0.90 ± 0.84	2.06 ± 1.52	0.61* ± 0.53	1.47 ± 0.64	1.07 ± 0.83

Table 5 Means and standard deviations of baseline and end of supplementation metabolic variables

VARIABLE	PHASE 1 PECTIN BASELINE	PHASE 1 PECTIN END	PHASE 1 PLACEBO BASELINE	PHASE 1 PLACEBO END	PHASE 2 PECTIN BASELINE	PHASE 2 PECTIN END	PHASE 2 ACETATE BASELINE	PHASE 2 ACETATE END
ACETATE (µmol/l)	50.65 ± 28.49	90.52* ± 43.14	44.20 ± 17.07	42.96 ± 21.55	37.81 ± 9.52	67.97* ± 25.95	37.63 ± 16.31	54.31* ± 12.12
PFA (mmol/l)	0.39 ± 0.03	0.31* ± 0.02	0.33 ± 0.03	0.43* ± 0.09	0.48 ± 0.06	0.40* ± 0.03	0.59 ± 0.05	0.45* ± 0.04
TP (g/l)	68.55 ± 7.99	66.84 ± 6.33	65.51 ± 5.26	64.09 ± 6.33	72.36 ± 4.04	75.21 ± 6.84	71.69 ± 4.52	71.52 ± 3.26
ALBUMIN (g/l)	47.23 ± 8.31	47.79 ± 2.96	47.46 ± 1.63	45.36 ± 6.10	45.53 ± 5.35	45.48* ± 2.69	43.09 ± 3.16	45.50 ± 2.12

VARIABLE	PHASE 1 PECTIN BASELINE	PHASE 1 PECTIN END	PHASE 1 PLACEBO BASELINE	PHASE 1 PLACEBO END	PHASE 3 PECTIN BASELINE	PHASE 3 PECTIN END	PHASE 3 ACETATE BASELINE	PHASE 3 ACETATE END
INSULIN (µU/ml)	10.95 ± 6.85	11.25 ± 6.34	17.14 ± 12.17	17.98 ± 13.21	18.55 ± 13.16	13.83 ± 7.34	8.96 ± 5.19	7.61 ± 4.78
GLUCOSE (mmol/l)	3.98 ± 0.34	3.72 ± 0.38	3.99 ± 0.59	3.96 ± 0.64	4.29 ± 1.52	4.00 ± 0.61	3.58 ± 0.39	3.78 ± 0.34
IR	4.29 ± 2.70	4.16 ± 2.38	7.16 ± 5.94	7.46 ± 6.02	9.40 ± 5.00	5.77 ± 3.77	3.22 ± 1.87	2.93 ± 1.93
PFA/ALB (X 10^2)	8.26 ± 0.43	6.49* ± 0.59	6.95 ± 0.27	9.48* ± 0.80	10.50 ± 0.44	8.78* ± 0.63	13.69 ± 0.88	9.89* ± 0.50

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The results of the above experiments will now be discussed briefly.

BODY WEIGHT AND BODY MASS INDEX (BMI) CHANGES

As is evident form Table 2, no significant changes in body weight or BMI

were observed in any of the groups during phase 1. The acetate

supplement (phase 2), however, caused a decrease (from 88.16 ± 12.35

kg to 83.09 ± 10.80 kg) in body weight. Although this decrease may not

be of statistical significance, it can be clinically significant in the cases of

those subjects who lost weight.

HAEMORHEOLOGICAL AND HAEMOSTATIC VARIABLES

As is evident from Table 3, pectin supplementation for both groups during

both phases caused a significant decrease in the clot lysis time,

Macromolucular-Protein-Complex-(MPC), clot fibrin content, Haemoglobin

(Hb), plasma viscosity, and a significant increase in fibrin clot compaction,

mass length ratio from turbidity (μT) and clot permeability.

Except for a significant decrease in the plasma viscosity in the placebo

group during phase 1 (from 1.80 ± 0.09 to 1.70 ± 0.07 cP), no other

changes were observed in this group.

It is furthermore clear from Table 3 that acetate supplementation caused a significant decrease in Haematocrit (Ht), Hb, plasma viscosity, MPC, clot fibrin content and clot lysis time, while significant increases were measured in clot compaction and permeability. Although the change in fibrinogen was

5   not significant, it is worthy to note that acetate supplementation caused a 11.2 % decrease in the total plasma fibrinogen concentration of the group.

LIPID CHANGES

As appears from Table 4, pectin supplementation caused significant

10   decreases in total cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), High Density Lipoprotein Cholesterol (HDL-C), and Apoprotein A (ApoA), Apoprotein B (ApoB), Lipoprotein (a) (Lp(a)), Tribarbituric Reactive Substances of Malondealdehyde (TBARM) and in hydrogen peroxide (H_2O_2) during phase 1. HDL-C was significantly increased during phase 2.

15

It is also apparent that ApoA decreased substantially in the placebo group. A significant decrease in ApoB was also measured. No other changes were significant.

20   It therefore appears that acetate supplementation caused a substantial decrease in TC, ApoA, ApoB, TG, and H_2O_2 while a significant increase in the %HDL-C was also evident.

METABOLIC VARIABLES

As appears from Table 5, which reflects the mean (SD) changes in some metabolic variables of both groups during both phases, pectin supplementation caused a significant increase in acetate levels and a

5   significant decrease in Free Fatty Acid (FFA) levels and ratio of FFA/albumin.

Except for a significant increase in the ration of FFA/albumin, no other significant changes were found in the placebo group.

10

It is also clear that acetate supplementation caused a substantial increase in acetate levels, and a significant decrease in FFA and ratio of FFA/albumin.

15   **EXAMPLE 2**

The effect of the acetate on the fibrin clot structure was further determined by in vitro studies and the results and a discussion thereof are given below.

ACETATE AND FIBRIN CLOT STRUCTURE

The effect of different concentrations of acetate on fibrin clot structure

properties (n = 5 each variable tested), is reflected in Table 6.

5

Table 6   The effect of different concentrations of acetate on fibrin clot structure
properties (n=5 for each variable tested)

[Acetate] (μmol/L)	Permeability (x 10^{11}cm^2)	μT (daltons/cm x 10^{12})
0	90.67 ± 8.00	14.92 ± 0.15
75	110.4 ± 5.17*	17.44 ± 0.20*
100	118.0 ± 6.03*	17.95 ± 0.22*
150	134.0 ± 5.02*	19.51 ± 0.17*

15

* differ significantly from 0 μmol/L acetate (p<0.05; Student t-test)

It is evident from Table 6 that as the acetate concentration increased

progressively from 0 μmol/L to 75, 100 and 150 μmol/L, the permeability

20   increased accordingly.   Fibre thickness from turbidity (μT) increased

significantly.   The clot lysis time decreased substantially, indicating

enhanced fibrinolysis with progressive acetate concentrations.   These

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changes in network characteristics do not arise from altered fibrinogen conversion because fibrin content did not alter substantially in the concentration range of the acetate tested. These findings probably indicate that the fibrin in the presence of acetate shows increased lateral

5   polymerization. Therefore a greater amount of fibrin is incorporated into the major network and the cross linking in the network is different to that of the control network.

The effect of different concentrations of acetate on clot fibrin content and sample viscosity (n = 5 for each variable tested) is reflected in Table 7 and the relation between fibrin network lysis and acetate concentrations is depicted in Figure 1.

5

Table 7   The effect of different concentrations of acetate on clot fibrin content and sample viscosity (n=5 for each variable tested)

[Acetate] (μmol/L)	Clot [FIBRIN] (g/L)	Lysis time (t½ / minutes)
0	1.35 ± 0.05	148.50 ± 2.50
75	1.36 ± 0.03	140.25 ± 2.23 *
100	1.37 ± 0.07	129.15 ± 1.66 *
150	1.39 ± 0.05	123.29 ± 2.02 *

* differ significantly from 0 μmol/L acetate (p < 0.05; Student t-test)

Figure 1:   Lysis by streptokinase of fibrin networks developed with

different concentrations acetate (n = 5 for each concentration

tested)
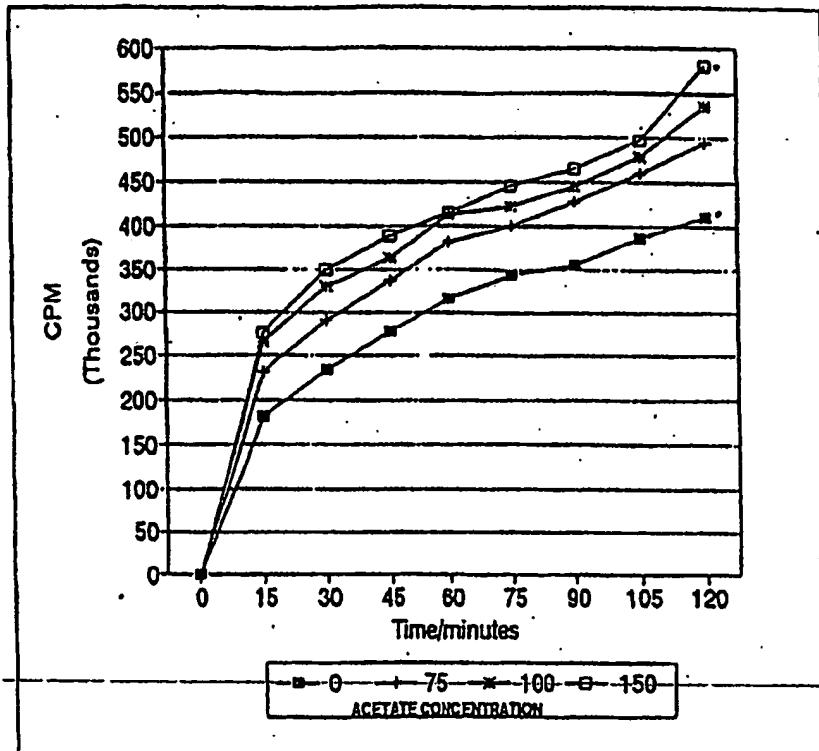
Referring to Table 7 and Figure 1, the lysis rate of radioactive-labelled fibrin

clots in the presence of different concentrations of acetate were quantified

by measuring released I^{125} in the medium over a determined time period. It

therefore appears that progressive acetate concentrations enhanced

fibrinolysis.

Referring to Figure 2, the kinetics of network growth were subsequently

investigated by continuously recording changes in turbidity at 608 nm,

during network development under identical experimental conditions. As

depicted in Figure 2, progressive increase in acetate enhanced the entire

5   kinetics. The lag phase became shorter, the increase in turbidity was faster

and the equilibrium turbidity was proportionally increased.

Figure 2:   Turbidity curve of fibrin formation in the presence of different

acetate concentrations

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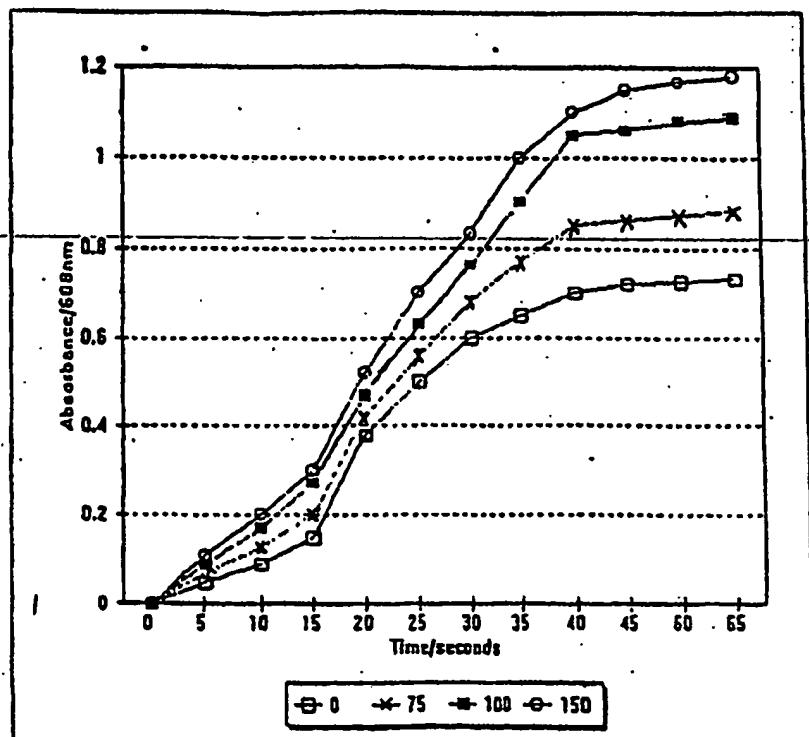
ACETATE AND LIPID PEROXIDATION

The effect of acetate on peroxidation of blood lipids *in vitro* (n = 5 for each measurement) is reflected in Table 8 and the relationship between the inhibition of peroxidation and acetate concentration is depicted in figure 3.

5

Table 8   The effect of acetate on peroxidation of blood lipids *in vitro* (n=5 for each measurement)

10

[Acetate] (μM)	[Hydroperoxide] x 10⁻⁶ M	% Inhibition
0.00 mmol/L	8.41 ± 0.20	0
0.05 mmol/L	4.46 ± 0.15 *	46.97
0.10 mmol/L	3.70 ± 0.22 *	56.01
0.20 mmol/L	3.04 ± 0.23 *	67.11
0.30 mmol/L	2.26 ± 0.16 *	73.12

15

* differ significantly from 0 μmol/L acetate (p<0.05; Student t-test)

20

Figure 3: The relationship between inhibition of peroxidation and acetate

concentration *in vitro*
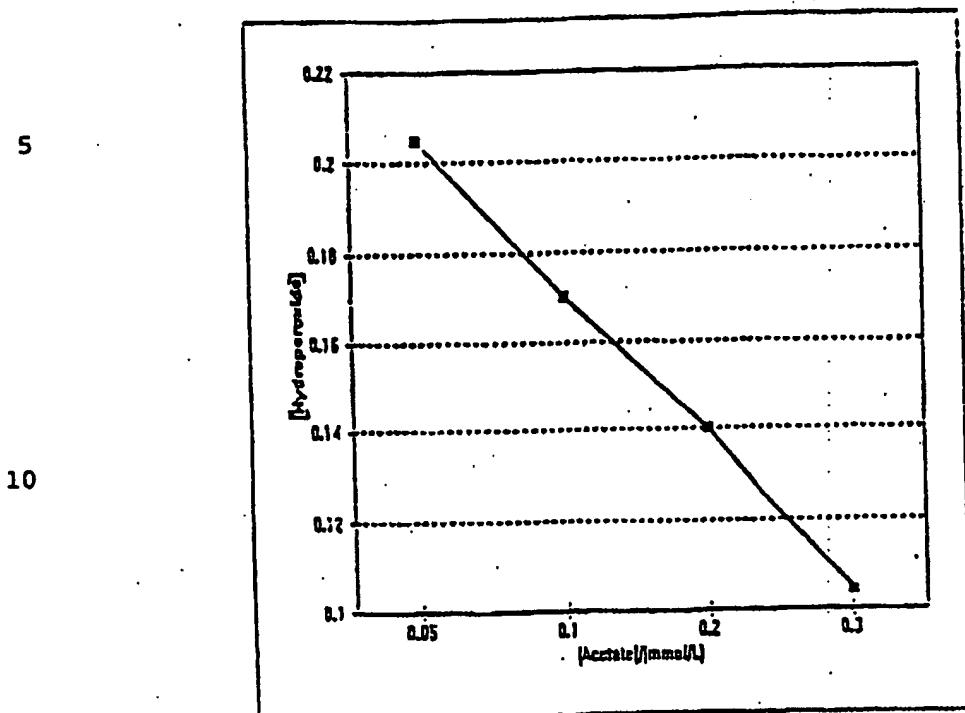
From Table 8 and Figure 3 it appears that there exists a linear analogy

between the extent of free radical inhibition and acetate concentration. A

46.97, 56.01, 67.11 and 73.12 % inhibition of free radical formation was

caused by 50 μM, 100 μM, 200 μM and 300 μM of acetate, respectively.

All these changes were significant (p <0.05). However, the graph of

Figure 3 suggests that acetate does not inhibit peroxidation in full. From

linear regression analysis, it seems that minium inhibition may cause a

56.12 % decrease of peroxidation *in vitro* (r = 0.98; m = -0.836). The

results showed that pectin supplementation caused a 49 % decrease in free radical content, which corresponds to an acetate concentration of 70 μM, if related to this *in vitro* study. This value is within physiological range. It is however, important to realize that the Cu^{2+} concentration used to

5   induce oxidation, is a drastic measurement, causing spuriously high rates of oxidation.

NOVEL EFFECTS

Pectin supplementation caused no substantial changes in plasma fibrinogen

10   levels. However, significant differences were found in the characteristics of networks developed in plasma of the pectin group. Networks were more permeable and had lower tensile strength. Their fibrin content decreased. markedly. A decrease in fibrin content partially explains some of the altered network characteristics due to altered fibrin(ogen) conversion. These

15   findings indicate that lateral polymerization was enhanced and a greater amount of fibrin was thus incorporated into the major fibre network. The increased major network fibre diameter is reflected in the turbidimetric measurement as shown in Figure 2. Fibrin fibre thickness seems to be determined by kinetics of its growth and differences in fibre diameter have

20   been attributed to the kinetics of fibrin(ogen) breakdown and subsequently fibrin fibre assembly. It is known that mass-length ratio of fibrin fibre is determined by the rates of generation of the fibrin monomer and that of its

assembly into fibrin fibre. When thrombin is added to fibrinogen, the fibrin monomer is generated according to the relative amounts of enzyme and substrate.

5   Turbidimetric changes represented by the lag phase, phase of increasing turbidity and the equilibrium phase, collectively represent the breakdown of fibrinogen to fibrin monomer; the initial aggregation of monomer to protofibrils; and the growth of protofibrils to an opaque network. The lag phase corresponds to the time required for the overall action of thrombin on

10   fibrinogen until the appearance of turbidimetrically detectable fibrin and includes the enzymatic breakdown of fibrinogen and the initial aggregation to protofibrils. The fibrinogen solution forms a gel during the early part of the second phase during which turbidity rises rapidly. The resulting increased thickness of fibres decreases the total contour length of the fibres

15   thus increasing the permeability. Networks with fibres of increased thickness and permeability are less resistant to lysis. Increased clot compaction also denotes a decrease in the tensile strength of fibrin. Increase in permeability and decrease in tensile strength indicates a smaller degree of cross linkage of fibres within the network.

20

The changes in fibrin network characteristic (μT and clot lysis time) were directly associated with the changes in plasma acetate levels.

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Acetate supplementation did not cause a significant change in plasma
fibrinogen levels, but a tendency of an 11.2 % decrease was observed in
this group. Significant differences were also found in the characteristics of
fibrin networks developed in plasma. These results were also observed
5   with the results of the pectin group. Changes in clot structure properties
were also associated with the changes in acetate levels. These results
strongly suggest that the effect of pectin on clot structure characteristics
were mediated by acetate.

10   Progressive amounts of acetate were used *in vitro* to investigate the
possibility that acetate may directly be responsible for changes of fibrin clot
structure characteristics *in vivo*, and rule out the effect of other possible
changes that occurred in the plasma medium. The results indicated that
acetate directly influence fibrin clot structure properties in the same manner
15   as during pectin and acetate supplementation. Increasing amounts of
acetate caused significant changes in the clot characteristics.

Although it is known that dietary fibre can modify lipid metabolism in man,
no effects of fibre or fibre components or metabolites on lipid peroxidation
20   have previously been reported. During the experiments, pectin
supplementation caused a significant decrease of 49% in the hydrogen
peroxide content of blood lipids. This effect was concomitant with a

POSSIBLE MECHANISMS

The results showed that both acetate and pectin *in vivo* induce alterations in network characteristics. However, pectin and acetate *in vivo* also showed significant effects on some other metabolic variables. Plasma is an aqueous mixture of proteins, lipids, carbohydrates, amino acids, salts and other substances. A change in any of these constituents of plasma would directly be reflected in the characteristics of fibrin networks. It would therefore seem that acetate and pectin can modify network characteristics by a combination of its effect on metabolism (modulating mechanism), possible direct effects (steric exclusion, etc.), and altered fibrin conversion (kinetic mechanism).

The mechanism underlying these differences is not clear at present, but in the investigation with artificially-added acetate the reagents were added only a few minutes before developing the network. The changes induced are thus from a direct effect of acetate on fibrin. Therefore it appears that in the presence of acetate added in this fashion, the networks developed simulated changes observed in network characteristics of both acetate and pectin supplemented subject plasma. This indicates that acetate may directly be responsible for partial changes in fibrin network characteristics.

decrease in total cholesterol. The change in lipid peroxides was directly associated with the change in TC and acetate levels.

Acetate supplementation caused a significant decrease in the free radical content of blood lipids. This effect was concomitant with a decrease in total cholesterol. The change in free radical concentration was directly associated with the change in TC and acetate levels.

The direct effect of acetate on lipid peroxidation was performed *in vitro* to rule out the effect of significant decreases in TC as reported for the acetate and pectin intervention results. The results showed that progressive amounts of acetate *in vitro* decreases the susceptibility of lipoproteins against free radical attack.

A clinically significant, but statistically insignificant decrease in body weight of 5.07 kg of the acetate supplemented subject group was observed. It was previously showed that acetate inhibits food intake in sheep. The acetate effect can therefore possibly be ascribed to be through direct mechanisms and a decrease in food intake. No weight reduction were measured in the pectin supplemented subject group. The weight loss with acetate supplementation probably contributed to the lowering of TC and TG.

The physiochemical nature of acetate defines the behaviour of this acid in living organisms. Molecules (such as acetate) of compounds contain O-H groups are attracted to each other by intermolecular force caused by the difference in the electronegativity of oxygen and hydrogen atoms. This gives acetate the ability to form hydrogen bonds between O-H, H-F, H-Cl and H-N. Hydrogen bonding is the key factor determining the characteristics of acetate in solution. There are two types of hydrogen bonding, intramolecular and intermolecular. Intermolecular bonding may be a link to the effects of acetate on fibrin clot structure *in vitro* and *in vivo*. Fibrinogen is a very large molecule with an array of different bonds. It is not impossible for acetate to form hydrogen bonds with the fibrinogen molecule, having both O-H and H-N groups. This may have steric effects on the fibrinogen molecule, causing a change in fibrinogen-thrombin interaction, which will consequently lead to an altered clotting process. This should lead to alterations in fibrin clot structure.

Both pectin and acetate decreases peroxidation of blood lipids *in vivo*. Excluding acetate, no other measured variable could explain this anti-oxidative effect of pectin and acetate *in vivo*. The underlying mechanism is not clear. From the *in vitro* results it seems that acetate inhibits lipid peroxidation directly. This indicates that pectin fermentation produces substances (acetate) with anti-oxidant properties. This may be direct

evidence that acetate protects against lipid peroxidation by inhibiting the release of free radicals, rather than protecting the blood lipids against them.

5 It will be appreciated that short chain fatty acids, such as acetic acid, or pharmaceutically acceptable salts, derivatives or precursors thereof, in a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestines of a mammal, but soluble and digestible in the colon of such mammal, could be used as a pharmaceutical agent for the prevention or treatment of any of the following

10 conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity and the use thereof. It will be appreciated further that such short chain fatty acids can further be used in methods for the treatment or prevention of any one

15 or more of said conditions in mammals.

It will be appreciated still further that there are no doubt a large number of variations in detail possible with the invention as hereinbefore described without departing from the scope and/or spirit of the appended claims.

20

CLAIMS

1.   A pharmaceutical agent for the prevention or treatment of any of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity, comprising a short chain fatty acid, or a pharmaceutically acceptable salt, derivative or precursor thereof, in a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but digestible or soluble in the colon of a mammal.

2.   A pharmaceutical agent according to claim 1 wherein the pharmaceutically-acceptable salt of the short chain fatty acid is the calcium salt thereof.

3.   A pharmaceutical agent according to claim 1 or claim 2 wherein the short chain fatty acid comprises acetic acid.

4.   A pharmaceutical agent according to any one of the preceding claims wherein the protective coating comprises a natural or synthetic resin such as shellac.

5.   A pharmaceutical agent according to claim 4 which comprises calcium acetate in the form of a capsule, tablet or pill coated with such a resin.

6.   A pharmaceutical agent according to claim 5 which comprises between 0,1 grams and 100,0 grams of the acetate.

7.   A pharmaceutical agent substantially as herein described and exemplified.

8.   A method for the treatment or prevention of any one or more of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity, including the step of administering to the colon of a mammal an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof.

9.   A method according to claim 8 wherein the agent is administered to the colon, via the digestive track of the mammal.

10.   A method according to claim 8 or claim 9 wherein the

       pharmaceutically acceptable salt is the calcium salt of the short chain

       fatty acid.

5   11.   A method according to any one of claims 8 to 10 wherein the short

              chain fatty acid is acetic acid.

                                    \

       12.   A method according to any one of claims 8 to 11 wherein the agent

              is administered in a pharmaceutically acceptable protective coating

10   which is resistant to digestion and solution in the stomach and small

              intestine of a mammal, but digestible or soluble in the colon of a

              mammal.

       13.   A method according to any one of claims 8 to 12 wherein the agent

15   is administered to a human being in an amount of between 0,1 gram

              and 100,0 gram at least once a day.

       14.   A method for the treatment or prevention of conditions in mammals

              substantially as herein described and exemplified.

20

15. Use of an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof, in a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a

5   mammal, but digestible or soluble in the colon of a mammal, in a method for the treatment or prevention of any one or more of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of

10   free fatty acids and obesity.

16. Use according to claim 15 of an agent wherein the pharmaceutically acceptable salt is the calcium salt of the short chain fatty acid.

15 17. Use according to claim 15 or claim 16 of an agent wherein the short chain fatty acid is acetic acid.

18. Use according to any one of claims 15 to 17 of an agent wherein the protective coating comprises a natural or synthetic resin such as

20   shellac.

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19. Use according to any one of claims 15 to 18 of an agent wherein the agent is administered to a human being in an amount of between 0,1 gram and 100,0 gram at least once a day.

5   20. Use of an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof, in the manufacture of a medicament for use in a method for the treatment or prevention of any one or more of the following conditions in mammals: atherosclerosis, thrombosis, unwanted high

10 levels of free radicals, unwanted long fibrin clot lysis times, unwanted fibrin clot characteristics, unwanted high levels of free fatty acids and obesity, the medicament having a pharmaceutically acceptable protective coating which is resistant to digestion and solution in the stomach and small intestine of a mammal, but

15 digestible or soluble in the colon of a mammal.

21. Use according to claim 20 of an agent wherein the pharmaceutically acceptable salt is the calcium salt of the short chain fatty acid.

20 22. Use according to claim 20 or claim 21 of an agent wherein the short chain fatty acid is acetic acid.

23. Use according to any one of claims 20 to 22 of an agent wherein the protective coating comprises a natural or synthetic resin such as shellac.

5   24. Use of an agent comprising a short chain fatty acid or a pharmaceutically acceptable salt, derivative or precursor thereof substantially as herein described and exemplified.